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Latent class analysis reveals clinically relevant atopy phenotypes in 2 birth cohorts

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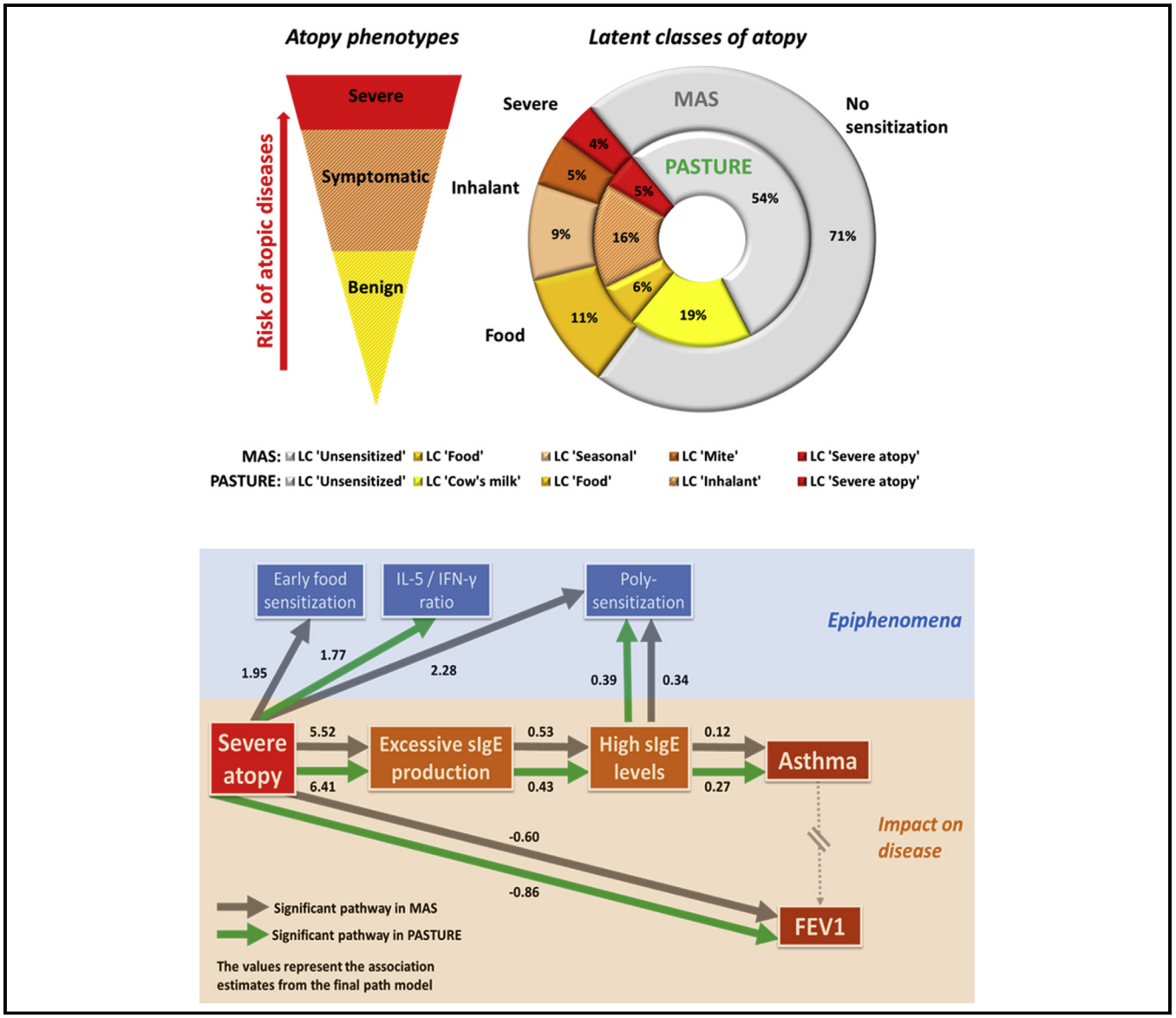
GRAPHICAL ABSTRACT



Background: Phenotypes of childhood-onset asthma are characterized by distinct trajectories and functional features. For atopy, definition of phenotypes during childhood is less clear.

Objective: We sought to define phenotypes of atopic sensitization over the first 6 years of life using a latent class analysis (LCA) integrating 3 dimensions of atopy: allergen specificity, time course, and levels of specific IgE (sIgE).

Methods: Phenotypes were defined by means of LCA in 680 children of the Multizentrische Allergiestudie (MAS) and 766 children of the Protection against allergy: Study in Rural Environments (PASTURE) birth cohorts and compared with classical nondisjunctive definitions of seasonal, perennial, and food sensitization with respect to atopic diseases and lung function. Cytokine levels were measured in the PASTURE cohort.

Results: The LCA classified predominantly by type and multiplicity of sensitization (food vs inhalant), allergen combinations, and sIgE levels. Latent classes were related to atopic disease manifestations with higher sensitivity and specificity than the classical definitions. LCA detected consistently in both cohorts a distinct group of children with severe atopy characterized by high seasonal sIgE levels and a strong propensity for asthma; hay fever; eczema; and impaired lung function, also in children without an established asthma diagnosis. Severe atopy was associated with an increased

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IL-5/IFN-γ ratio. A path analysis among sensitized children revealed that among all features of severe atopy, only excessive sIgE production early in life affected asthma risk. Conclusions: LCA revealed a set of benign, symptomatic, and severe atopy phenotypes. The severe phenotype emerged as a latent condition with signs of a dysbalanced immune response. It determined high asthma risk through excessive sIgE production and directly affected impaired lung function. (J Allergy Clin Immunol 2017;139:1935-45.)

Key words: *Atopy, IgE, sensitization, asthma, lung function, cytokines, severe atopy, atopic diseases, latent class analysis, unsupervised clustering, path analysis, epidemiology*

Asthma and atopy often manifest concomitantly before school age, but the interrelation of both phenomena remains obscure, possibly because both conditions can result from a multitude of individual pathologies with complex interferences that blur the entire picture. In the case of asthma, wheezing phenotypes have been identified and consolidated by using data-driven approaches.[1-3] However, these approaches are currently only emerging for atopy classification.

Because of cosensitizations, categorization by allergen specificity or type of sensitization is ambiguous and leads to overlapping groups, such as food, inhalant perennial, or inhalant seasonal sensitization.[4] Other approaches applying disjunctive categories mainly rely on temporal patterns, focusing on age of onset,[5-9] longitudinal trends,[10] persistence of IgE sensitization,[11,12] or refer to multiplicity of allergen specificities (ie, monovalent vs polyvalent sensitization).[13-18] However, it has been pointed out that all the above approaches are susceptible to investigator bias.[19] This issue can be overcome by data-driven, unsupervised statistical methods, such as latent class analysis (LCA). Until now, these approaches focused on allergen specificities at one[20] or several[19,21,22] time points but did not consider the strength of sensitization as assessed by IgE levels.

We appraised this omission a shortcoming given the well-known disease relevance of IgE levels[23] and therefore included this dimension in our analysis. We applied LCA to 2 rather different birth cohorts: the urban Multizentrische Allergiestudie (MAS) cohort and the rural Protection against allergy: Study in Rural Environments (PASTURE) study. The aim of this analysis was to compare LCA-derived classification with classical definitions of atopy based on carrier polymer system (CAP) classes and to relate both systems to manifestation of asthma, allergic diseases, cytokine expression, and lung function. Finally, we sought to integrate the various aspects of atopy in a path model for asthma and lung function.

Abbreviations used
CAP: Carrier polymer system
LC: Latent class
LCA: Latent class analysis
MAS: Multizentrische Allergiestudie
PASTURE: Protection against allergy: Study in Rural Environments
sIgE: Specific IgE

METHODS

Study design and population

Both birth cohorts were set up to study the development of childhood asthma and allergies. MAS recruited 1314 healthy mature infants born in 1990 in 5 German cities (Berlin, Düsseldorf, Freiburg, Mainz, and Munich).[24] Of those, 499 had risk factors for atopy (ie, increased cord blood IgE levels [≥0.9 kU/L] or at least 2 atopic family members). PASTURE recruited 1133 children in 2002 to 2005 from rural areas in 5 European countries: Austria, Finland, France, Germany, and Switzerland.[25] Children of mothers living on family-run livestock farms were assigned to the farm study group. The reference study group comprised children of mothers from the same rural areas but not living on a farm. Both studies were approved by the ethics committees of the participating institutions, and written informed consent was obtained from the children's parents or guardians.

Atopic sensitization (specific IgE in serum samples)

In the MAS cohort serum samples were obtained from the children at 1, 2, 3, 5, 6, and 7 years of age. Levels of specific IgE (sIgE) antibodies to food allergens (cow's milk, egg white, soy bean, and wheat) and inhalant allergens (the house dust mite *Dermatophagoides pteronyssinus*, cat dander, mixed grass, birch pollen, and dog dander from age 3 years on) were determined with ImmunoCAP (Phadia, Freiburg, Germany). Soybean was excluded from the analyses because it was not measured in PASTURE for all time points, and dog dander was excluded because of the lack of measurements at years 1 and 2.

In the PASTURE cohort sIgE for 6 food and 13 common inhalant allergens was assessed in cord blood samples and at the ages of 12, 54, and 72 months in peripheral blood by using the semiquantitative Allergy Screen test panel for atopy (Mediwiss Analytic, Moers, Germany) in a central laboratory.[4] Because of common cross-reactivity and low frequencies of some specificities, the original 19 specificities were combined into 9 categories finally entered into the LCA: grass pollen (rye pollen or grass pollen mix), tree pollen (alder, birch pollen, or hazel pollen), cat, dog, mites (*Dermatophagoides pteronyssinus* or *D farinae*), hen's egg, cow's milk, wheat flour, and nuts (peanut or hazelnut). In the MAS cohort the categories of nuts and dog were not available.

Questionnaires

In the MAS cohort, at each follow-up visit at the age of 1, 3, 6, 12, 18, and 24 months and then yearly within 4 weeks of the child's birthday up to age 7 years, parents were interviewed for asthmatic and atopic symptoms and disease, diet, development, and psychological aspects. From age 5 years onward, questions relating to wheeze corresponded to the International Study of Asthma and Allergies in Childhood core questions. In the PASTURE cohort questionnaires were administered at the end of pregnancy and when the children were 2, 12, 18, 24, 36, 48, 60, and 72 months of age to obtain information on frequencies of wheeze, parental atopic status, and environmental exposures with a focus on farming and nutrition.[4] Variable definitions were harmonized between both studies. Lifetime asthma was defined as a physician's diagnosis of asthma at least once per lifetime as reported by the parents at age 6 years; children with no diagnosis of asthma and no current wheeze in the last 12 months served as control subjects. Hay fever was defined as parent-reported rhinitis symptoms ever or a physician's diagnosis of hay fever or allergic rhinitis ever at age 6 years. Atopic dermatitis was defined as a physician's diagnosis of atopic eczema at least once per lifetime, as reported by the parents at age 6 years; children with no diagnosis of atopic eczema and no atopic eczema in the last 12 months were the control subjects.

Lung function measurements

At age 7 years in the MAS cohort in 801 children[6] and at age 6 years in the PASTURE cohort in 799 children,[3] FEV_1 was measured and z-standardized.[26]

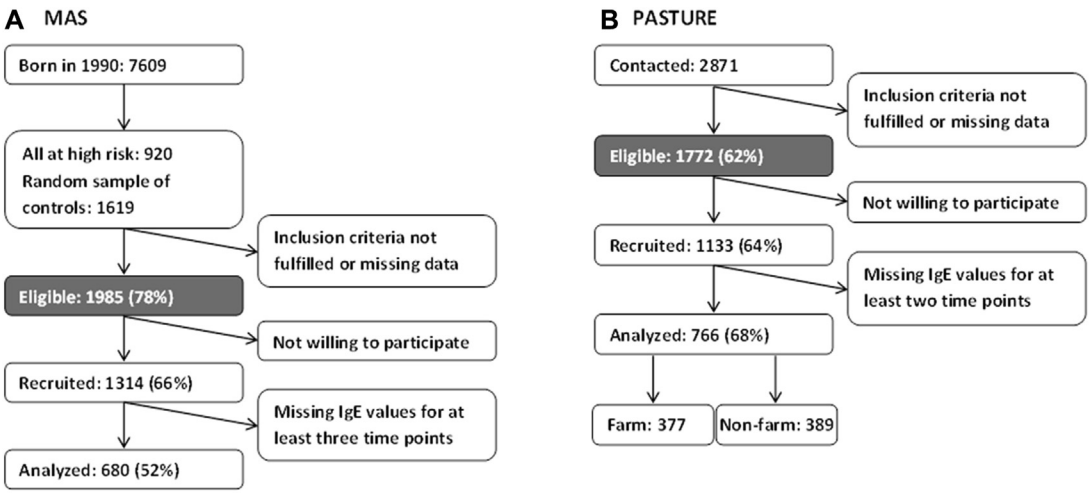

FIG 1. **A** and **B,** Selection of study populations.

Cytokine assessment

In the PASTURE cohort whole-blood supernatants from 6-year-old children were collected after 48 hours of stimulation with 5 ng/mL phorbol 12-myristate 13-acetate and 1 μg/mL ionomycin. IL-5 and IFN-γ levels were measured in supernatants by using the multiplexed Cytometric Bead Array (BD Biosciences, San Jose, Calif) in Marburg, Germany. The detection limit was 0.01 pg/mL, and values of less than were replaced by 0.001 pg/mL in 17 (IL-5) and 11 (IFN-γ) subjects. Cytokine concentrations were standardized to peripheral blood leukocyte counts (Sysmex KX-21N Blood Cell Analyzer; Sysmex, Kobe, Japan) and z-transformed.

Statistical analysis

Children with missing sIgE data for at least 3 of 6 (MAS) or 2 of 4 (PASTURE) measurement time points were excluded. For all other children, missing sIgE values were imputed by using multiple linear imputation of the continuous sIgE values in 20 replicates. Categorical variables were created from the imputed continuous variables for sIgE levels with the following categories: sIgE levels of less than 0.35 kU/L, 0.35 kU/L or greater to 0.7 kU/L or less, 0.7 kU/L or greater to less than 3.5 kU/L, and 3.5 or greater corresponding to CAP classes. In the PASTURE cohort the lowest category was again split at 0.2 kU/L because of the comparably lower sIgE values and a lower detection limit of the measurement method.

For each imputed data set, an LCA based on categorized sIgE values between birth and year 6 was performed, assigning subjects to classes by their highest posterior probabilities,[27] and each subject was assigned to the latent class (LC) in which it was classified in the majority of the 20 replications (for further details, see the Methods section in this article's Online Repository at www.jacionline.org). The retrieved LCs were arbitrarily labeled according to their key features to enhance recognition.

Classical definitions of atopy were defined as being sensitized to a specific allergen or groups of allergens (seasonal, perennial, or food allergens) at a specific CAP class at a specific time point irrespective of sensitizations to other allergens. The LCs were compared with these classical definitions with respect to true- and false-positive rates by using receiver operating characteristic curves.

Associations of outcomes with potential determinants were calculated by using linear or logistic regression. Effect estimates are presented with 95% CIs as odds ratios (ORs) for dichotomous outcomes and β-estimates for linear continuous outcomes, such as lung function parameters. All regression analyses were adjusted for center and in the PASTURE cohort additionally for study group. Control subjects used in the regression models for LCA were subjects assigned to the LC unsensitized, and for classical definitions, control subjects were children without any sensitization at CAP class 1 at the respective time point. Statistical analyses were performed with SAS 9.4 software (SAS Institute, Cary, NC) and Mplus 7 software (Muthén & Muthén, Los Angeles, Calif).

RESULTS

The analysis population consisted of 680 MAS cohort children (52% of 1314 at recruitment; Fig 1, *A*) and 766 PASTURE cohort children (68% of 1133; Fig 1, *B*) with complete or imputed sIgE values, who did not differ from the excluded children with respect to sensitization status at any age (see Table E1 in this article's Online Repository at www.jacionline.org). The LCA revealed solutions with 3 to 6 classes, with the best Akaike information criterion values for the 5-class solutions in both studies (see Table E2 in this article's Online Repository at www.jacionline.org). The distribution of LCs across study centers was rather homogenous in both studies (see Fig E1 in this article's Online Repository at www.jacionline.org).

As shown in Fig 2, the largest classes containing 71% (MAS) and 54% (PASTURE) of all children were characterized by the absence of sensitization and consequently labeled as unsensitized. One MAS class and 2 PASTURE classes included mainly children with sensitization to food allergens. The MAS cohort children in the food class were predominantly monosensitized to cow's milk or hen's egg; in the PASTURE cohort the larger class was sensitized only to cow's milk, and the other class was sensitized to food allergens beyond cow's milk. The remaining classes represented mainly inhalant sensitization. In the PASTURE cohort one class included children with sensitization predominantly to either seasonal or perennial inhalant allergens. The corresponding MAS cohort children were grouped into 2 classes with either sensitization to seasonal or mite allergens. The smallest class within each study was termed severe atopy for its specific features, as explained below.

A hallmark of the severe atopy class was sensitization predominantly to seasonal allergens up to CAP class 3, with a steep increase in the prevalence of sensitization before year 4 or 5. Food cosensitization occurred in the majority of this LC (MAS, 88%; PASTURE, 67%) and mite cosensitization occurred in a relevant proportion (MAS, 31%; PASTURE, 26%) at year 6 and CAP class 2. In the severe atopy class of the MAS cohort, food

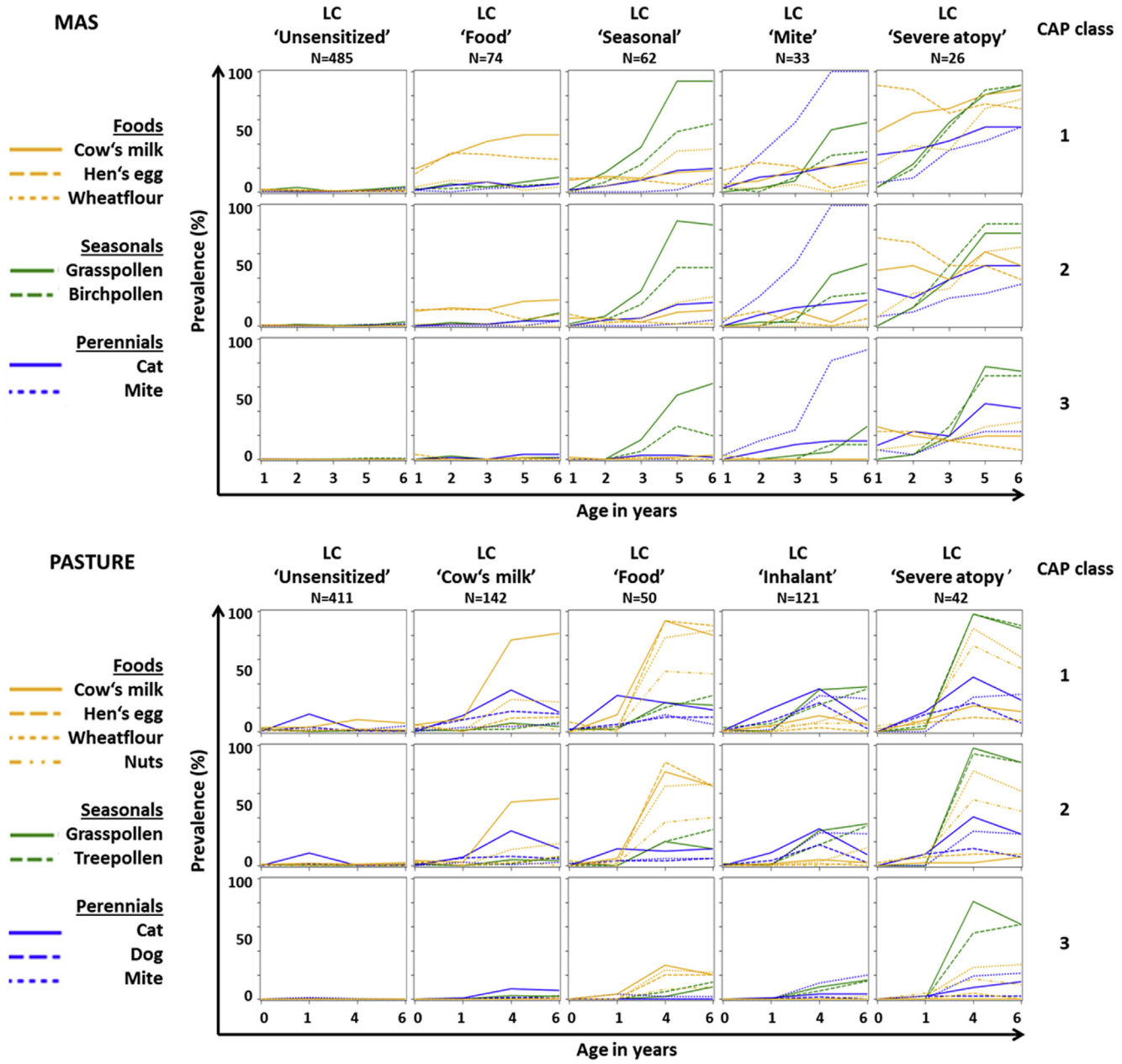

FIG 2. LCs of atopy as characterized by allergen specificity, time course, and sIgE levels.

cosensitization was very common already at year 1 (81%, CAP class 2). In the PASTURE cohort food sensitization at year 1 occurred in 22% when considering a cutoff level of 0.2 kU/L. Taken together, LCA grouped mainly for allergen specificity (food vs inhalant classes), for strength of sensitization, and partially for temporal patterns.

LCs are mutually exclusive and integrate information across CAP classes and over various time points, whereas classical definitions of sensitization, such as sIgE to any inhalant or any food allergens, can overlap and depend on the underlying CAP class and time point of measurement. Although both systems were comparable at the most suitable time points and CAP cutoff levels as determined by receiver operating characteristic curves (see Fig E2 and Table E3 in this article's Online Repository at www.jacionline.org), their associations with disease manifestations diverged in several instances (Fig 3 and see Fig E3 in this article's Online Repository at www.jacionline.org). In both studies sIgE values to any food allergens overestimated associations with asthma- and health-related conditions when compared with the food LCs. Conversely, LC severe atopy was associated more with these conditions compared with sIgE against any inhalant allergens, even at CAP class 3. The associations of disease risk with the respective LCs were paralleled by those of parental atopy (see Fig E4 in this article's Online Repository at www.jacionline.org). A sensitivity analysis (see Figs E5 and E6 in this article's Online Repository at www.jacionline.org)

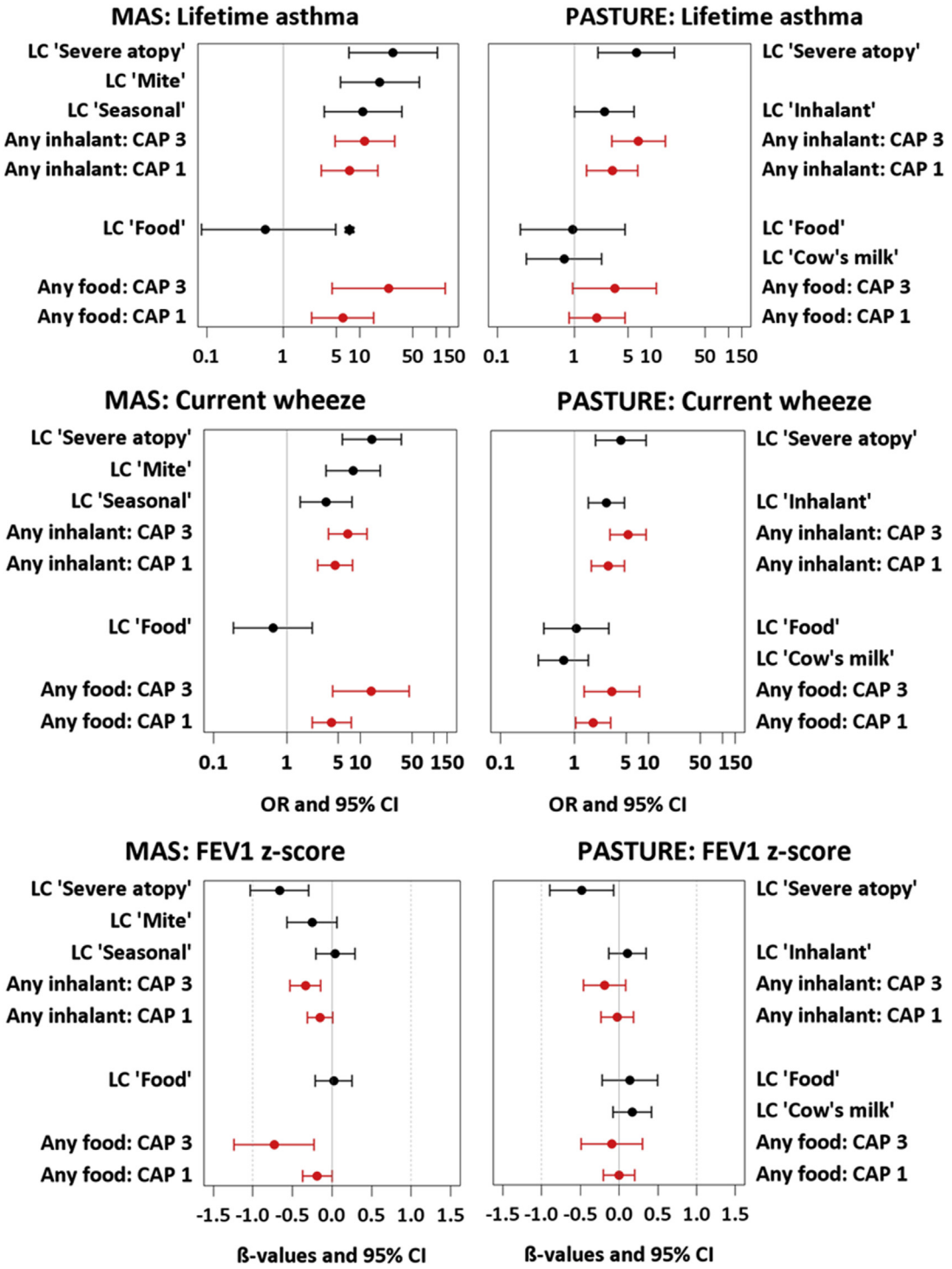

FIG 3. Associations of asthma-related conditions with LCs and classical definitions of atopic sensitization at age 6 years. *Because there was no case of lifetime asthma in this LC, we calculated a conservative estimation of the odds ratio *(OR)* based on one case of asthma in this LC, which was simulated at random. *Black point estimates with error bars* mark the LCs as reference, red marks the classical definitions as a comparison.

revealed that each of the 3 dimensions of allergen specificity, sIgE levels, and time course contributed importantly to the composition and disease relevance of the respective LCs.

Based on disease relevance, the LCs were grouped within 3 atopy phenotypes (Fig 4): LCs related to food sensitization represented a benign phenotype without any disease relevance, and

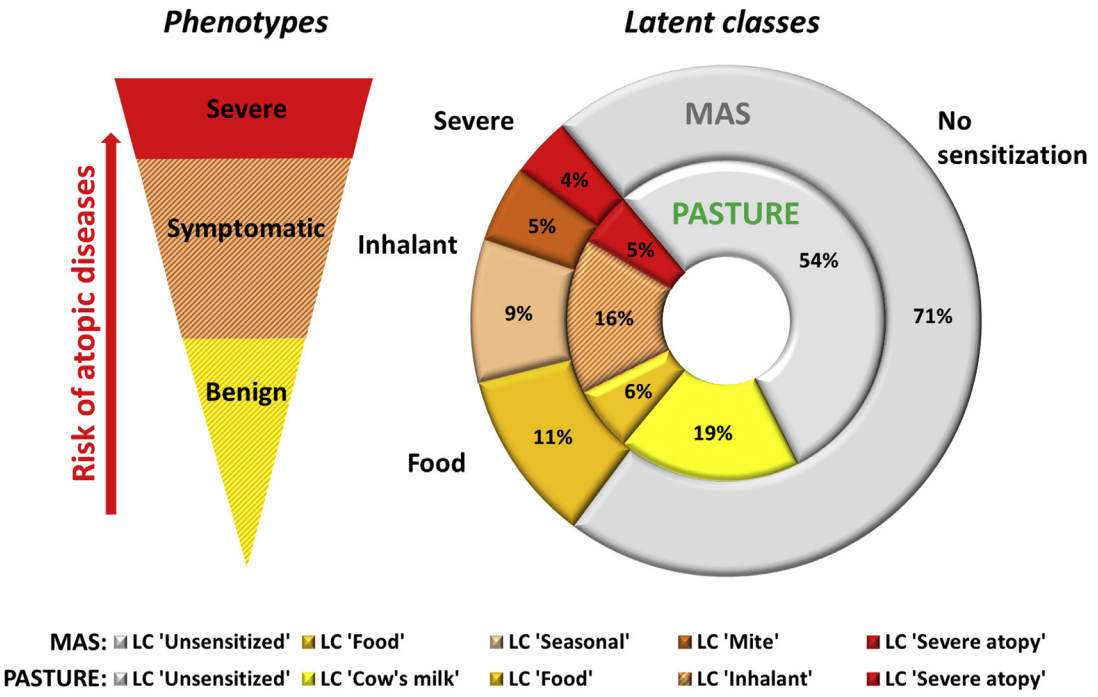

FIG 4. Atopy phenotypes in relation to the distribution of LCs in both populations.

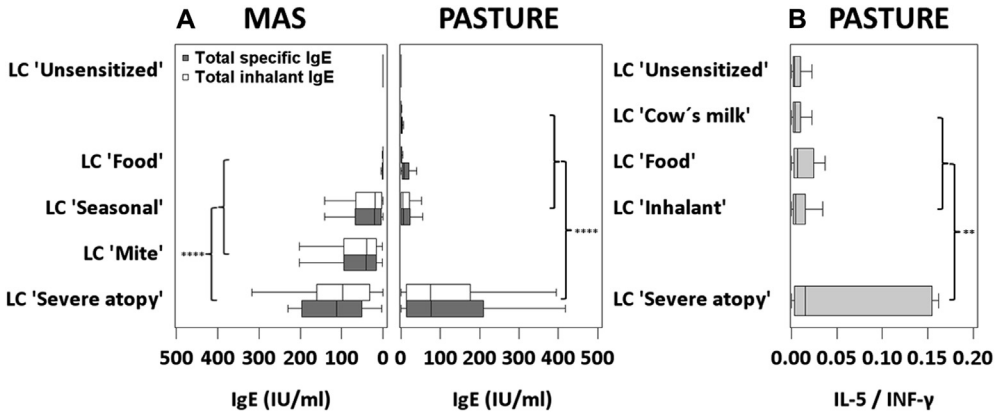

FIG 5. Absolute sIgE levels **(A)** and ratio of IL-5 to IFN-γ expression **(B)** at age 6 years.

LCs related to inhalant sensitization corresponded to a symptomatic phenotype with risk of asthma yet normal lung function. In contrast, the LC severe atopy was characterized by impaired lung function and a much higher propensity for atopic disease.

To better understand the singular phenomenon of severe atopy and to contrast it with benign and symptomatic sensitized children, we assessed the biologically relevant features of atopy. Although the LCA discriminated well between oligovalent and polyvalent sensitization, polyvalence was not specific for severe atopy but also characterized food sensitization in the PASTURE cohort (see Fig E7 in this article's Online Repository at www.jacionline.org). However, a unique feature of severe atopy consisted in high levels of sIgE to inhalant, particularly seasonal, allergens ($P < .0001$, Fig 5, *A*, and see Fig E8 in this article's Online Repository at www.jacionline.org). This resulted from an excessive increment in sIgE levels in the first 3 to 4 years (and a milder trend in subsequent years) compared with the weak increase in symptomatic and benign atopy, particularly for seasonal and food sIgE ($P < .0001$, Table I). Similarly, severe atopy differed from the other LCs with respect to the ratio of IL-5 over IFN-γ expression, thereby reflecting the activation of T_H2 rather than T_H1 subsets ($P < .01$; Fig 5, *B*).

To elucidate the mutual relation between severe atopy and the various features differentiating it from the benign and symptomatic phenotypes, we performed a path analysis (Fig 6). In both studies asthma was determined by severe atopy through an excessive increment in sIgE to seasonal allergens during the first 6 years and high sIgE levels at 6 years. Although including only 5% of all children, severe atopy explained 20% of all sensitized asthma cases. Early sensitization to food allergens, T_H2/T_H1 ratio, and

TABLE I. Increment in sIgE production comparing severe atopy with the other atopy phenotypes

		Seasonal sIgE		Food sIgE		Perennial sIgE	
Time period	**Study**	**β (95% CI)**	***P* value**	**β (95% CI)**	***P* value**	**β (95% CI)**	***P* value**
Early increase							
Year 0 to year 1	PASTURE	0.05 (−0.28 to 0.39)	.7491	0.69 (0.12 to 1.25)	**.0175**	0.30 (−0.50 to 1.09)	.4653
Year 1 to year 3	MAS	4.28 (2.97 to 5.60)	**<.0001**	3.29 (1.93 to 4.66)	**<.0001**	0.62 (−0.78 to 2.02)	.3843
Year 1 to year 4	PASTURE	7.25 (6.32 to 8.18)	**<.0001**	2.45 (1.55 to 3.35)	**<.0001**	1.29 (0.22 to 2.37)	**.0187**
Late increase							
Year 3 to year 6	MAS	2.47 (0.84 to 4.10)	**.0030**	3.78 (2.47 to 5.09)	**<.0001**	1.44 (0.01 to 2.87)	**.0483**
Year 4 to year 6	PASTURE	1.21 (−0.01 to 2.43)	.0524	−0.27 (−1.09 to 0.55)	.5193	1.06 (0.11 to 2.00)	**.0290**
Overall increase							
Year 1 to year 6	MAS	5.01 (3.33 to 6.68)	**<.0001**	4.65 (3.23 to 6.07)	**<.0001**	2.03 (0.09 to 3.97)	**.0404**
Year 1 to year 6	PASTURE	6.13 (4.99 to 7.27)	**<.0001**	1.17 (0.21 to 2.12)	**.0163**	1.79 (0.66 to 2.92)	**.0018**

The β estimates result from linear regression of the log-transformed sIgE values on severe atopy versus the other 2 atopy phenotypes within the respective time period adjusted for baseline sIgE values. Estimates remained stable after mutual adjustment for incremental increase of the other specificities. Values in boldface indicate statistical significance.

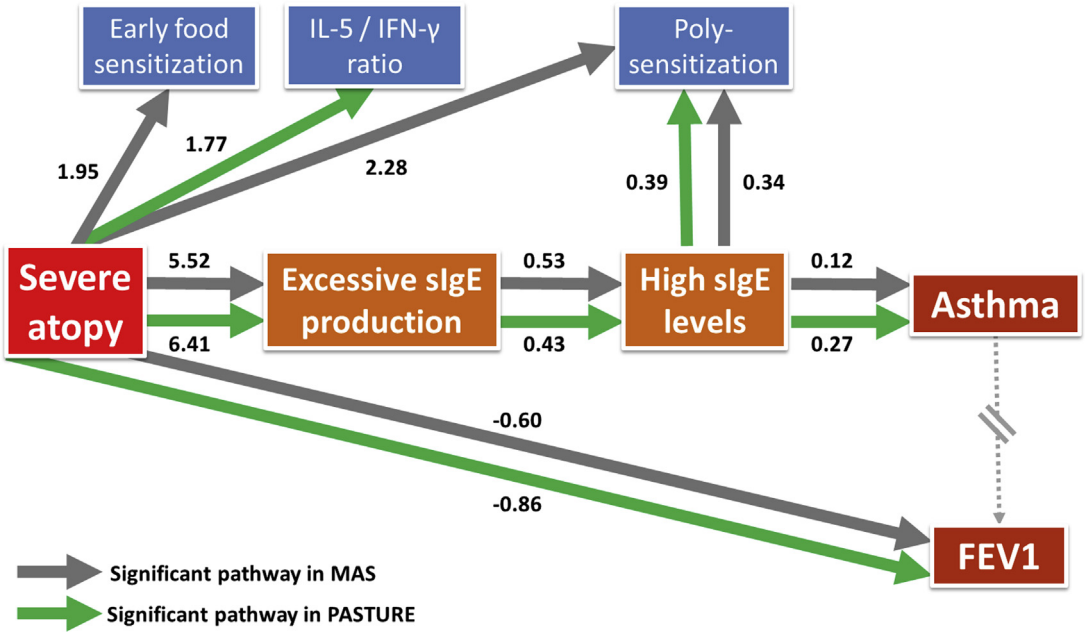

FIG 6. Path diagram comparing severe atopy with the other atopy phenotypes, including its features, lung function, and asthma in both populations. Excessive sIgE production is defined as incremental increase of seasonal sIgE production during the first 6 years. High sIgE levels are determined at age 6 years. Significant associations are shown by *solid arrows*. Absent associations are represented by *interrupted dotted arrows*. Values represent association estimates from the final model, including significant paths only.

polysensitization were similarly determined directly or indirectly by severe atopy but not related to asthma.

Similarly, as in atopic subjects also in the entire population of both cohorts, the inverse association of sIgE levels and FEV_1 was completely explained by severe atopy (change in estimate, 104%), as held partially true for the association of asthma and FEV_1 (change in estimate, 38%). This was not unexpected because the severe atopy class contained also a substantial proportion of children without current wheeze or an established asthma diagnosis but with FEV_1 values within the lowest decile (Fig 7).

DISCUSSION

Using LCA, we classified preschool children for sensitization patterns considering the 3 dimensions of allergen specificity, time course, and strength of sensitization. The resulting LCs were related to manifested atopic disease with higher sensitivity and specificity compared with classical definitions of sensitization. The food LCs of both cohorts emerged as a benign atopy phenotype without individual risk and family history of asthma. A symptomatic phenotype was found in the inhalant LCs with substantial risk of atopic diseases but without impaired lung function. The severe atopy phenotype represented by the LC of severe atopy comprised children with high sIgE levels to seasonal allergens, much stronger associations with atopic disease, and low FEV_1 values, even in those without an established asthma diagnosis.

A major advantage of this analysis was the comprehensive approach covering the first 6 years of life, with detailed information on various major allergen specificities at different levels. Missing values were successfully imputed, thereby providing a complete data set for 1446 children without observable selection

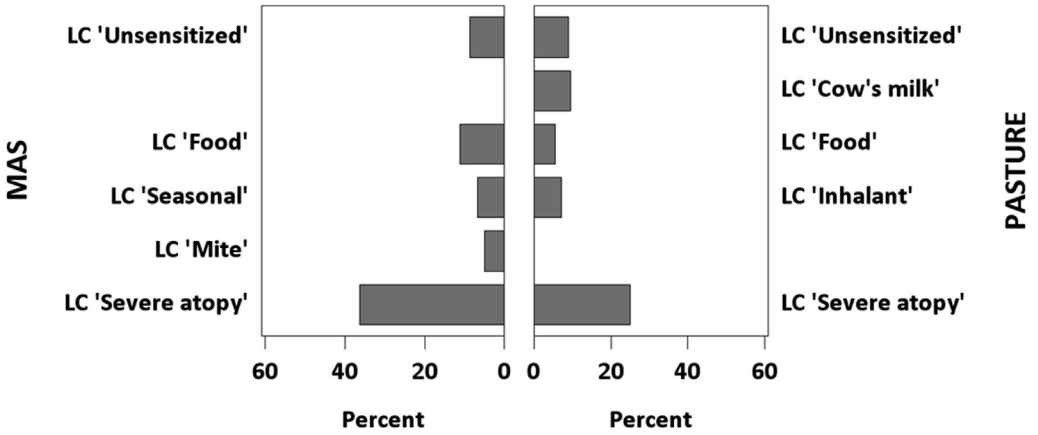

FIG 7. Proportion of nonasthmatic children with reduced lung function by LCs. Reduced lung function was defined as values in the lowest decile of the FEV_1 distribution.

from the originally recruited populations. A further strength was the replication of the main findings in 2 rather different birth cohorts.

Admittedly, not all LCs were fully congruent between the studies: the LC with monosensitization to cow's milk at low sIgE levels was specific for the PASTURE cohort and might be explained by the rather common consumption of cow's milk in this rural population. Correspondingly, in the MAS cohort a specific mite class emerged, reflecting the relevance of this allergen in an urban cohort. An additional characteristic of the MAS cohort was the higher proportion of early sensitization to food allergens in the LC of severe atopy, possibly resulting from the recruitment focus on children with increased sIgE levels in cord blood. Nevertheless, these peculiarities do not interfere with the core results of this analysis.

The role of sIgE in the manifestation of atopic diseases has long been discussed controversially. In 1989, Burrows et al[23] suggested a linear relation between total IgE levels and asthma risk. Ten years later, the question arose whether increases in total IgE levels were to some extent determined by specific wheeze phenotypes.[8] Soon thereafter, Illi et al[11] hypothesized that "an underlying condition drives both a certain pattern of sensitization and the development of childhood asthma." Later, the concept of multiplicity of sensitizations was introduced as a genuine risk factor for respiratory allergy.[13-19,28]

Against this background of conflicting hypotheses, we sought a unifying concept. Without providing any information on atopic disease, an LCA based on time course and levels of sIgE against food and inhalant allergens yielded a clear trichotomy with respect to manifestation, severity, and family history of atopic disease in both cohorts. Using classical definitions of atopy, such as sIgE levels to any food or any seasonal allergens, the respective associations were overestimated or underestimated, and the signals were diluted.

The detection of an innocent or benign atopy phenotype predominantly related to food sIgE is clinically relevant and suggests that children with asthma allocated to one of the benign food LCs should not be considered atopic asthma in epidemiologic studies. Rather, these children might experience nonatopic asthma and concomitantly happen to produce irrelevant food sIgE, as do many children without asthma.

Also, the distinction between symptomatic and severe atopy has vast implications: children with symptomatic atopy have a lower risk of asthma, hay fever, and eczema, and a less severe phenotype, as suggested by rather normal lung function parameters.

Severe atopy was characterized by specific and also unspecific features. With the LC of food in the PASTURE cohort, severe atopy shared polyvalent sensitization with 5 or more allergens (see Fig E7). Children with early food sensitization were allocated to both the severe atopy and food LCs with similar absolute counts, although at different proportions. Although early food sensitization can be seen as the first raised flag of severe atopy, it cannot serve as a specific predictor of this condition among sensitized children. However, a unique hallmark of severe atopy was the increased T_H2/T_H1 cytokine ratio at age 6 years (Fig 5, *B*). This emerging dysbalance might result from an initial T_H2 cell activation without subsequent resolution into "protective immunologic tolerance," as suggested by Rowe et al.[29] In addition to the specifically strong association with impaired lung function, severe atopy harbored a relevant proportion of children with FEV_1 values in the lowest decile but without an established asthma diagnosis. In practical terms this group of children might benefit from further clinical work-up and careful monitoring of sIgE increments within the first 3 to 4 years.

A further exclusive feature of severe atopy consisted in high sIgE levels, which followed a steep increase in seasonal sensitization particularly before age 3 to 4 years. This sharp increase was the only relevant longitudinal variation among the LCs and distinguished the current LCA for atopy from an earlier LCA for wheeze.[3] This earlier LCA was entirely determined by the time course of symptoms and produced a late-onset wheeze phenotype emerging only beyond age 3 to 4 years with strong associations with atopic sensitization, particularly severe atopy (see Fig E3). In this context it is noteworthy that the steep increase in sIgE levels within the severe atopy class preceded the first symptoms of the atopic late-onset wheeze phenotype. This temporal relationship in combination with the strength and specificity of the association of severe atopy with asthma and impaired lung function and the consistency of the findings between both studies argues in favor of a causal relationship.

To corroborate this assumption, we performed a path analysis contrasting severe atopy with benign and symptomatic atopy in regard to the above features. According to this analysis, the effect of severe atopy on asthma was completely mediated through the steep increase in sIgE levels and the resulting high sIgE levels.

Because this steep increase was seen for all sIgE specificities in severe atopy (Table I), one might hypothesize that excessive sIgE production is a generic phenomenon beyond any specific allergen.

This crucial role of uncontrolled sIgE production is indirectly supported by evidence from clinical studies showing an alleviating effect on childhood asthma symptoms by neutralizing sIgE with an anti-IgE antibody.[30,31] Vice versa, the pathway model might provide a suitable explanation for the efficacy of anti-IgE treatment. Additionally, severe atopy, or in practical terms a steep increase in sIgE levels until age 3 to 4 years, might serve as a selection criterion for children susceptible to anti-IgE therapy. Because severe atopy explains at least every fifth case of atopic asthma, a relevant share of children might profit from this therapeutic approach.

Moreover, severe atopy directly determined low FEV_1 values and explained the inverse association of FEV_1 and asthma, ultimately implying that poor lung function at age 6 years is not a feature of asthma unless it is related to severe atopy. In other words, poor lung function and excessive production of sIgE might result from the same latent phenomenon. This shared pathogenesis might point toward a local process of uncontrolled production of sIgE in the bronchial mucosa,[32] which again might be the target of future interventions.

The other features of severe atopy (ie, early food sensitization, an increased T_H2/T_H1 ratio, and polysensitization) emerged from the path analysis as epiphenomena without any proper effects on asthma risk. Rather, they might hint at an authentic latent phenomenon, which manifests with many faces.

Integrating temporal patterns, allergen specificity, and strength of sensitization in a data-driven approach, we found 3 phenotypes of atopy with respect to disease relevance. In contrast to benign and symptomatic atopy, severe atopy identified a circumscribed group of children with high sIgE values, pronounced disease risk, and poor lung function. Thus severe atopy as a latent phenomenon might correspond to the condition underlying both childhood asthma and sensitization patterns, as previously postulated by Illi et al.[11] The path analysis performed in atopic subjects now suggests a link between severe atopy and asthma through excessive sIgE production, particularly to seasonal allergens early in life, and might direct further research into the biologic fundamentals of atopy.

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Clinical implications: Atopic sensitization was classified into benign, symptomatic, and severe phenotypes. Severe atopic children were characterized by a strong propensity for atopic diseases mediated by excessive sIgE production early in life, and poor lung function, even in those without an established asthma diagnosis.

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METHODS

Statistical analysis

Multiple imputation was based on continuous sIgE levels from at least 4 of 6 time points in the MAS cohort (age, 1-7 years) and 3 of 4 time points in the PASTURE cohort (age, 0-6 years). Multiple linear imputation was performed in 20 runs, resulting in 20 data sets for each cohort, and then the continuous values for each data set were transformed into 4 ordinal CAP classes for the MAS cohort (<0.35, <0.7, <3.5, or ≥3.5 kU/L) and 5 ordinal CAP classes for the PASTURE cohort (1 additional CAP class <0.2 kU/L). At this step, data up to age 6 years were used in both studies for comparability. Finally, in the MAS cohort 35 four-stage variables representing 7 allergen specificities at 5 time points and in the PASTURE cohort 36 five-stage variables representing 9 allergen specificities at 4 time points were entered in the LCAs, which were performed for each of the 20 imputed data sets per cohort. For each LCA, subjects were assigned to classes based on their highest posterior probabilities. Each subject was assigned to its definite LC by the majority of the class memberships in 20 repeats. In addition, class membership was confirmed by visualizing sIgE prevalences in analogy to Fig 2.

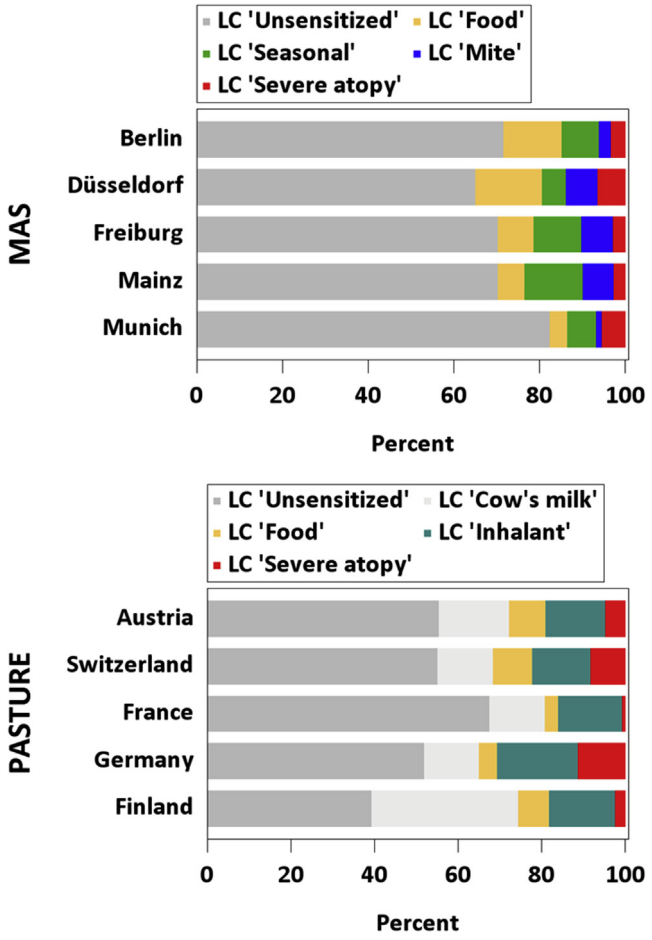

FIG E1. Distribution of LCs across study centers.

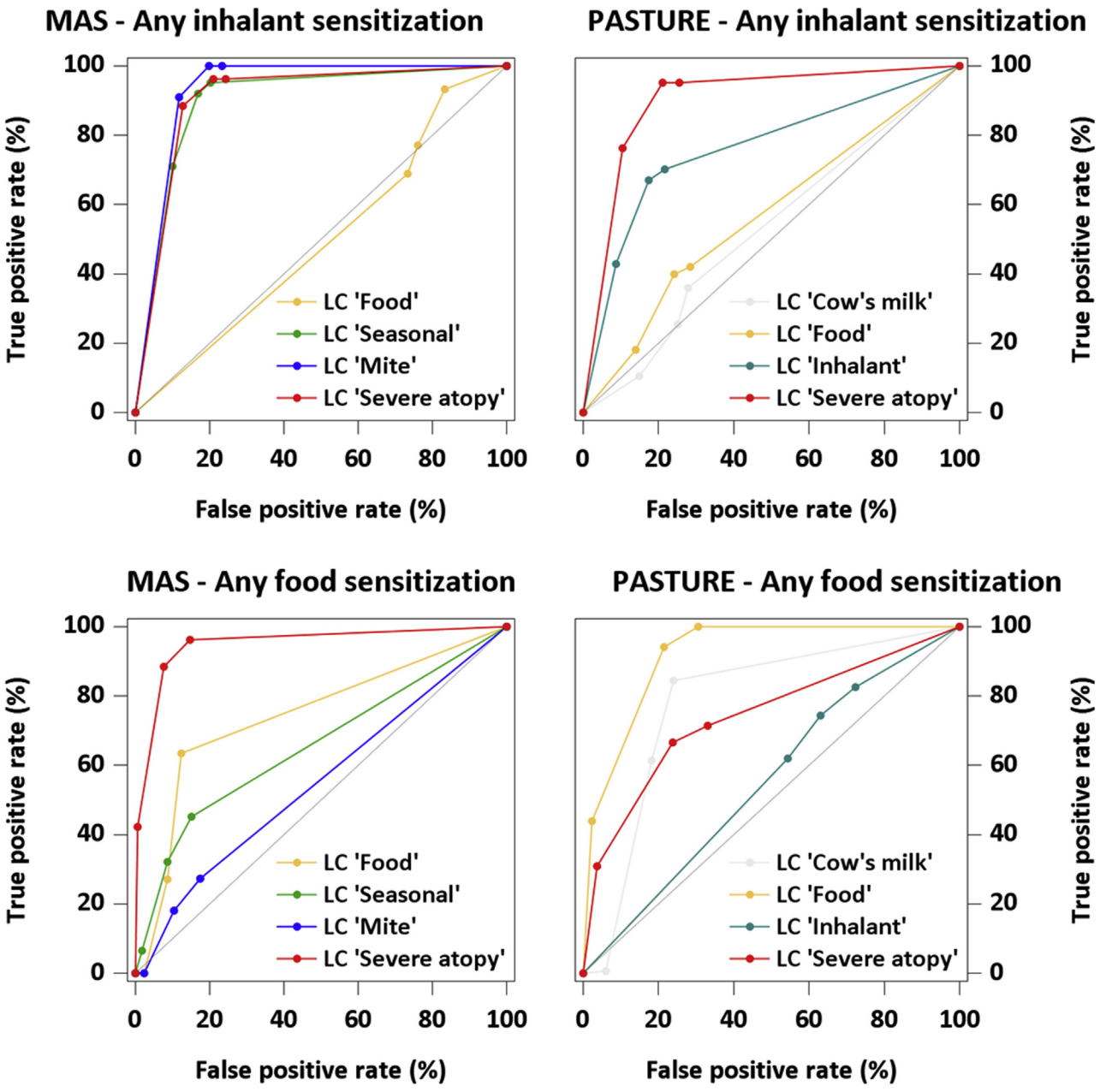

FIG E2. Prediction of LCs by classical definitions of sensitization at age 6 years. *Dots* mark the sensitization status (from right to left: unsensitized and CAP classes 1-3).

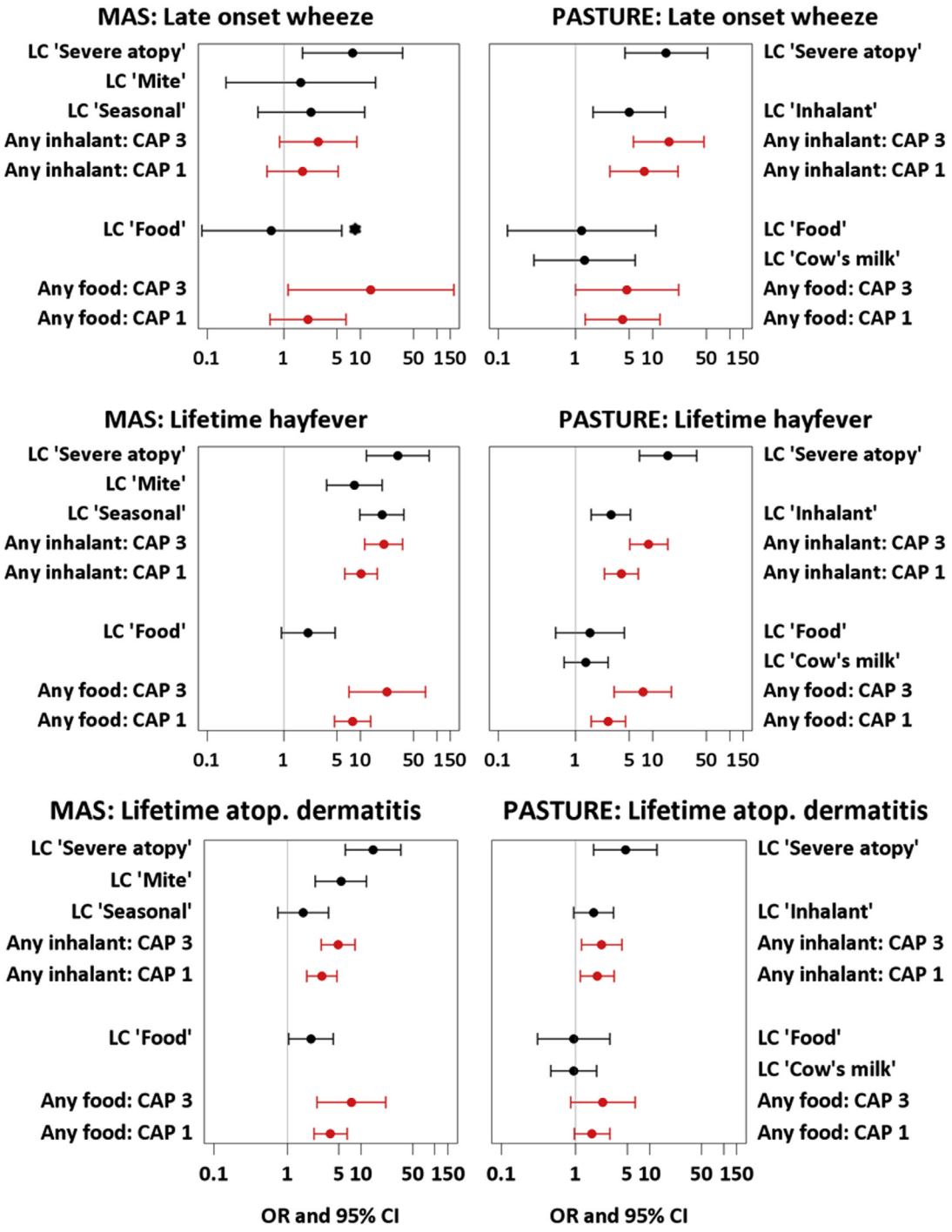

FIG E3. Associations of health conditions with LCs and classical definitions of atopic sensitization at age 6 years. *Because there was no case of late-onset wheeze in this LC, we calculated a conservative estimation of the OR based on 1 case of late-onset wheeze in this LC, which was simulated at random. *Black point estimates with error bars* mark the LCs as reference, and red marks the classical definitions as comparison. Late-onset wheeze was defined as described by Depner et al.[3]

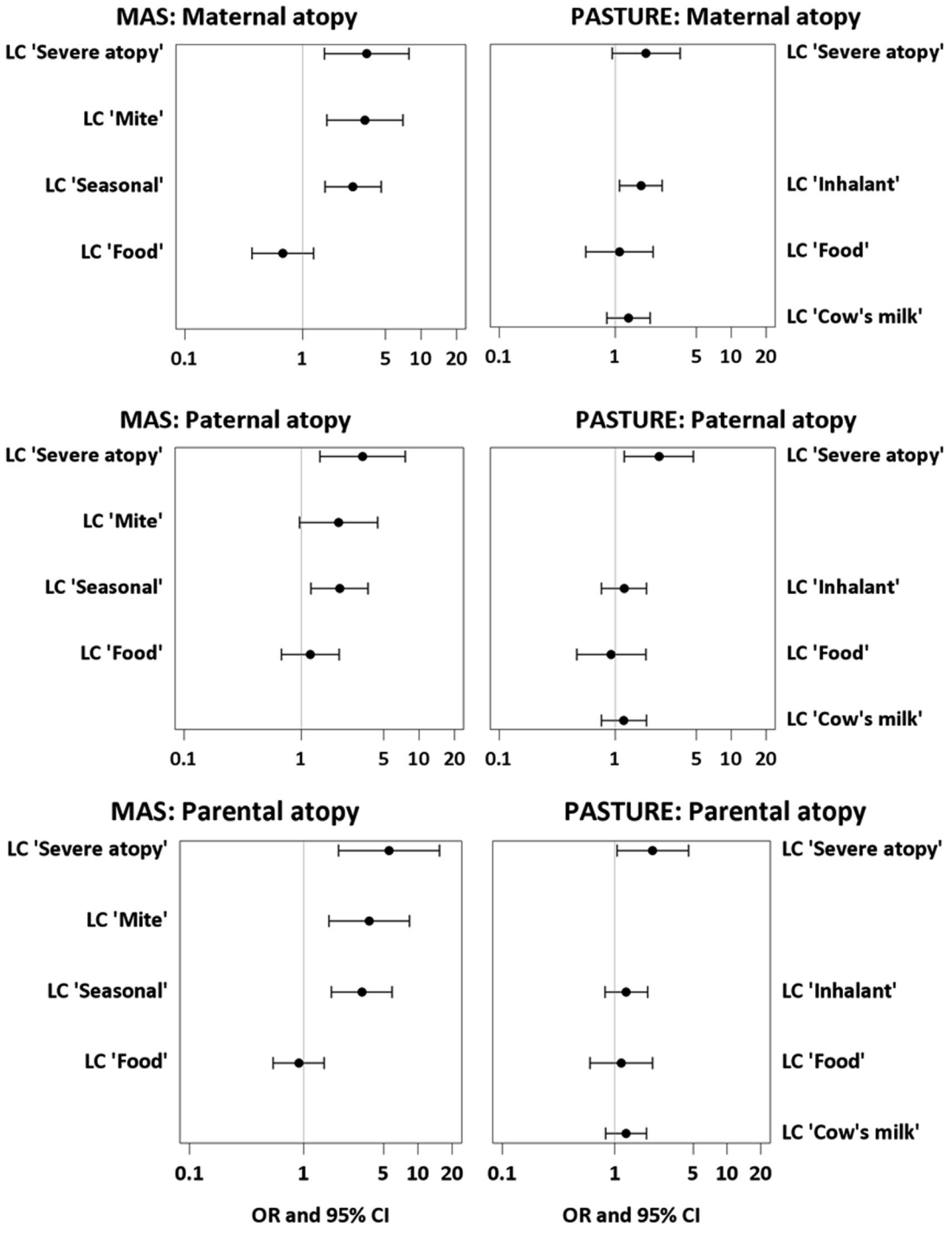

FIG E4. Associations of LCs with parental atopy.

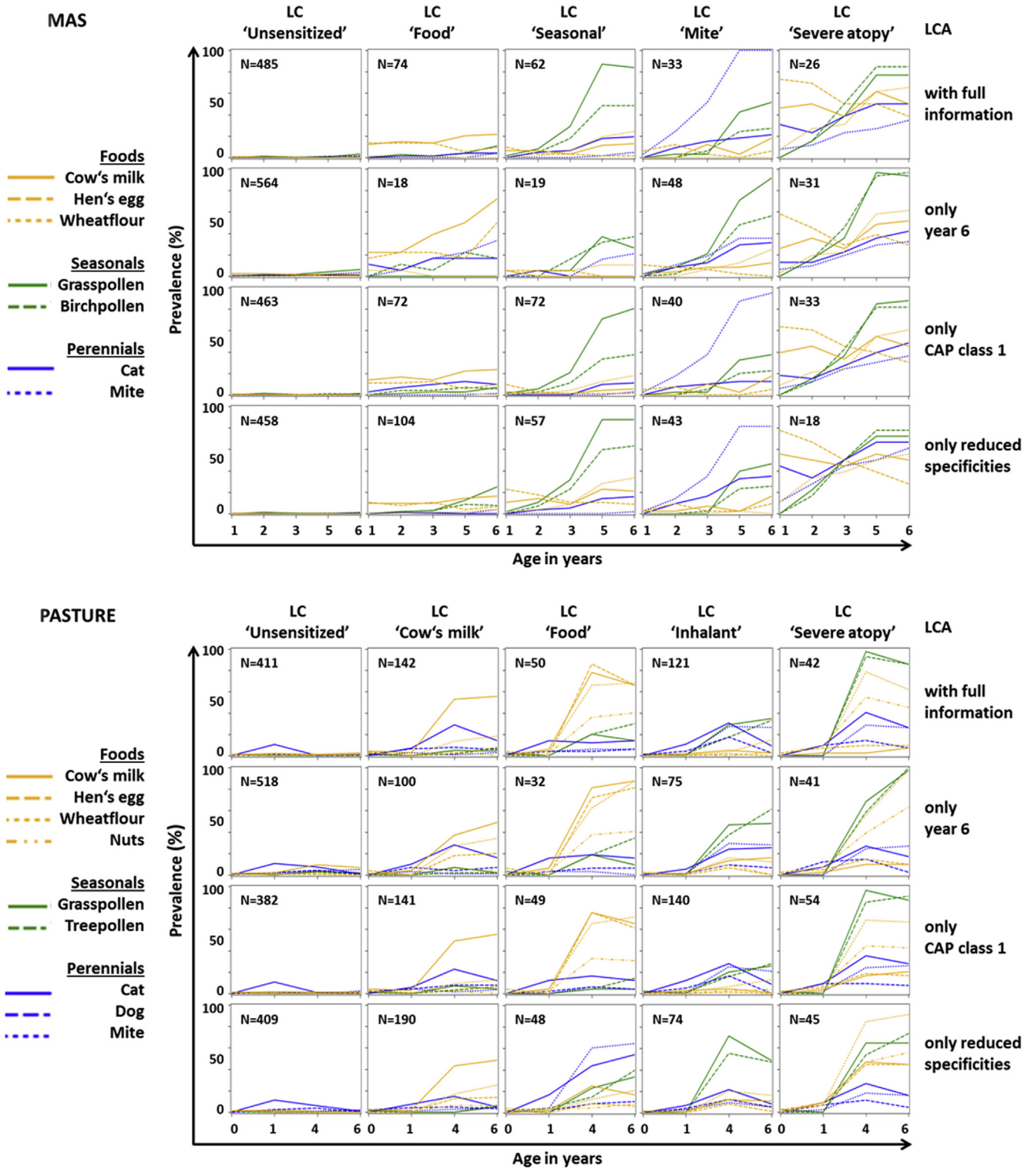

FIG E5. Sensitivity analyses omitting single dimensions of LCA.

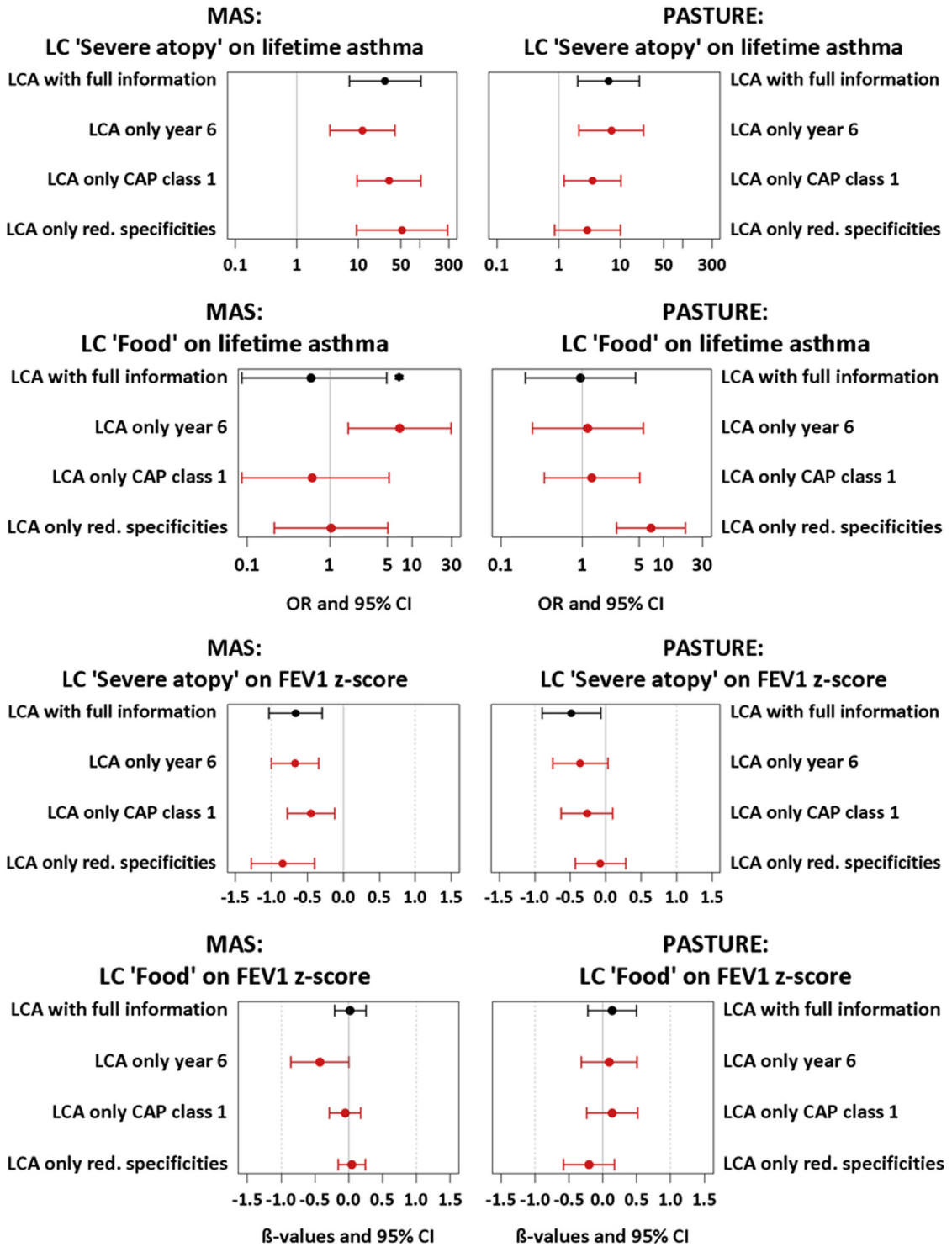

FIG E6. Comparing disease associations across all sensitivity analyses. *Because there was no case of lifetime asthma in this LC, we calculated a conservative estimation of the odds ratio *(OR)* based on 1 case of asthma in this LC, which was simulated at random. *Black point estimates with error bars* mark the LCs as reference, and red marks the classical definitions as a comparison.

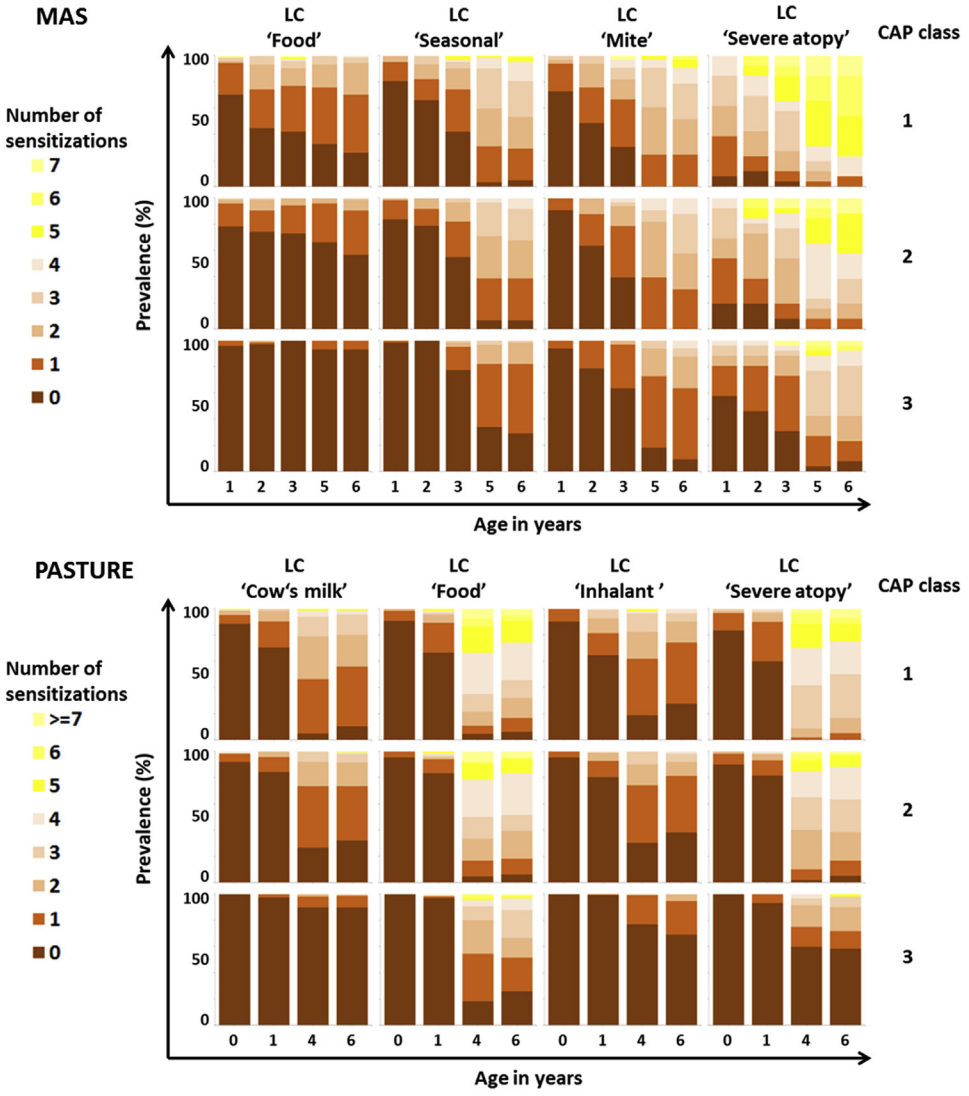

FIG E7. Number of sensitizations to different allergen specificities across LCs (CAP classes 1-3).

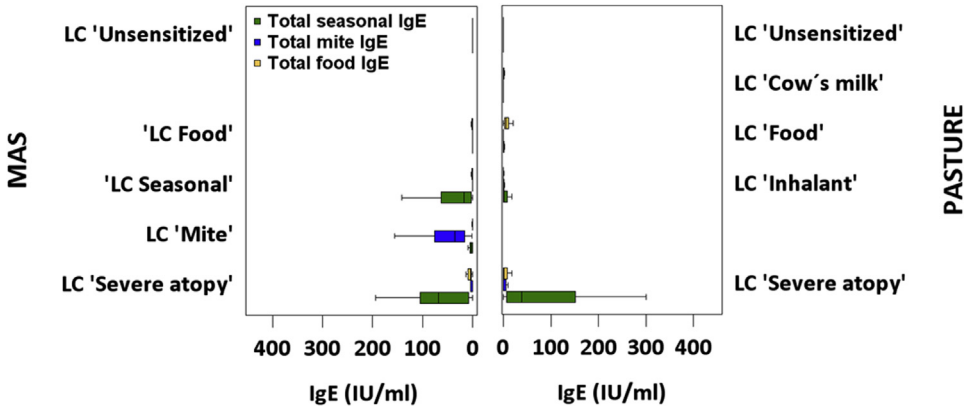

FIG E8. Absolute sIgE levels at age 6 years.

TABLE E1. Selection of study population

	MAS cohort						PASTURE cohort					
		Not included		Included				Not included		Included		
Variable		No.	Percent	No.	Percent	*P* value		No.	Percent	No.	Percent	*P* value
Center 1	Berlin	316	49.84	278	40.88	**.0011**	Austria	94	25.61	126	16.45	**.0003**
Center 2	Düsseldorf	52	8.20	109	16.03	**.0000**	Switzerland	84	22.89	158	20.63	.3847
Center 3	Mainz	101	15.93	111	16.32	.8466	France	46	12.53	157	20.50	**.0011**
Center 4	Freiburg	100	15.77	108	15.88	.9567	Germany	94	25.61	160	20.89	.0743
Center 5	Munich	65	10.25	74	10.88	.7106	Finland	49	13.35	165	21.54	**.0010**
High-risk group		229	37.06	253	37.76	.7936		—	—	—	—	—
Farming		—	—	—	—	—		153	41.69	377	49.22	**.0175**
Sex (female)		303	47.79	327	48.09	.9144		161	49.39	369	48.30	.7421
Family history of allergic disease		309	50.08	345	50.88	.7726		174	51.63	417	54.72	.3431
Maternal history of allergic disease		207	33.33	226	33.28	.9850		110	30.05	261	34.07	.1779
High parental education		362	88.08	531	87.05	.6264		307	87.46	701	91.87	**.0198**
At least 2 older siblings		76	11.99	89	13.11	.5406		112	30.52	267	34.86	.1475
Breast-feeding ever (in first year)		547	88.37	612	90.94	.1292		256	89.20	689	90.90	.4048
Environmental tobacco smoke		201	58.94	315	53.48	.1062		32	17.88	56	8.20	**.0001**
Doctor-diagnosed asthma at age 6 y		13	4.48	28	4.61	.9305		4	2.27	36	5.28	.0917
Sensitized to any allergen at birth (CAP class 1)		—	—	—	—	—		31	12.20	81	11.91	.9024
Sensitized to any allergen at age 1 y (CAP class 1)		28	13.66	90	16.70	.3107		60	28.17	204	28.10	.9841
Sensitized to any allergen at age 2 y (CAP class 1)		36	24.49	131	25.49	.8063		—	—	—	—	—
Sensitized to any allergen at age 3 y (CAP class 1)		29	24.79	133	26.71	.6713		—	—	—	—	—
Sensitized to any allergen at age 4 y (CAP class 1)		—	—	—	—	—		19	59.38	396	57.81	.8609
Sensitized to any allergen at age 5 y (CAP class 1)		48	40.34	180	34.16	.2026		—	—	—	—	—
Sensitized to any allergen at age 6 y (CAP class 1)		42	42.42	160	37.74	.3883		25	54.35	376	53.79	.9415

Absolute numbers and percentages (in parentheses) are shown. *P* values are derived from χ^2 tests. The 2 columns represent the excluded part of the entire population without complete sIgE data and the analysis population with complete sIgE data after imputation for the selected time points. Values in boldface indicate statistical significance.

TABLE E2. Model parameters of LCA

No. of classes	AIC	Entropy
MAS		
3	7,384 (7,361-7,406)	0.96 (0.95-0.96)
4	7,172 (7,151-7,192)	0.95 (0.95-0.96)
5	**7,064 (7,044-7,084)**	**0.97 (0.97-0.97)**
6	7,067 (7,047-7,088)	0.96 (0.96-0.97)
PASTURE		
3	14,632 (14,474-14,791)	**0.95 (0.94-0.96)**
4	14,444 (14,290-14,597)	0.92 (0.91-0.94)
5	**14,357 (14,202-14,511)**	0.93 (0.92-0.94)
6	14,382 (14,234-14,530)	0.93 (0.91-0.95)

Mean values of AIC and entropy are given with 95% CIs for 20 imputed data sets. Values in boldface indicate for the AIC the minimum value as indicator for the best model fit, and for the entropy the maximum value as indicator for the best model usefulness.

AIC, Akaike information criterion.

TABLE E3. Prediction of LCs by classical definitions of sensitization at age 6 years: AUC of receiver operating characteristic analyses with 95% CIs

LCs	Any inhalant sensitization	Any food sensitization
MAS		
LC food	49.52 (44.34-54.69)	74.13 (68.58-79.68)
LC seasonal	90.10 (86.74-93.45)	65.48 (58.92-72.04)
LC mite	93.22 (91.55-94.89)	54.87 (47.00-62.73)
LC severe atopy	90.70 (86.20-95.20)	94.89 (90.56-99.23)
PASTURE		
LC cow's milk	53.92 (49.40-58.43)	81.78 (78.74-84.82)
LC food	57.85 (50.26-65.44)	91.98 (89.72-94.25)
LC inhalant	77.49 (72.87-82.11)	55.45 (50.75-60.15)
LC severe atopy	90.67 (87.17-94.16)	76.08 (68.24-83.91)

AUC, Area under the receiver operating characteristic curve.